Enclosure to letter of September 3, 2004
International Patent Application No. PCT/EP04/00729
Applicant: Roche Diagnostics GmbH
Applicant's Ref.: 21581 WO-BUR

New Patent Claims

1. Method for the conversion of a cytosine base in a nucleic acid to an uracil base comprising the steps of

   a) incubating a solution comprising the nucleic acid for a time period of 1.5 to 3.5 hours at a temperature between 70 and 90 °C, whereby the concentration of bisulfite in the solution is between 3 M and 6.25 M and whereby the pH value of the solution is between 5.0 and 6.0 whereby the nucleic acid is deaminated, and

   b) incubating the solution comprising the deaminated nucleic acid under alkaline conditions whereby the deaminated nucleic acid is desulfonated.

2. Method according to claim 1,
   characterized in that
   in step a) the temperature is between 75 and 85 °C.

3. Method according to any of the claims 1 to 2,
   characterized in that
   the concentration of bisulfite is between 3.2 M and 6 M.

4. Method according to any of the claims 1 to 3,
   characterized in that
   the pH value of the solution is between 5.25 and 5.75.

5. Method according to any of the claims 1 to 4,
   characterized in that
   the time period is between 1.75 and 3 hours.

6. Method according to any of the claims 1 to 5,
   characterized in that
   the time period is between 2 and 3 hours.

7. Method according to any of the claims 1 to 6, characterized in that in step a) the temperature is 80 °C, the concentration of bisulfite is 5 M, the pH value of the solution is 5.5 and the time period is between 2 and 3 hours.

8. Use of a solution with a pH value between 5.25 and 5.75 comprising bisulfite in a concentration between 3 M and 6.25 M at a reaction temperature between 70 and 90 °C and optionally comprising hydroquinone in a reaction wherein a cytosine base in a nucleic acid is converted to an uracil base in the presence of bisulfite ions...

9. Use according to claim 8 wherein the concentration of bisulfite is between 3.2 M and 6 M.

10. Use according to any of the claims 8 to 9 wherein the pH value of the solution is 5.5 and wherein the concentration of bisulfite is 5 M.

11. Kit comprising a solution with a pH value between 5.25 and 5.75 comprising bisulfite in a concentration between 3 M and 6.25 M and optionally comprising hydroquinone.

12. Solution with a pH value between 5.4 and 5.6 and comprising bisulfite in a concentration between 3.5 M and 6.25 M and optionally comprising hydroquinone.

13. Solution according to claim 12 wherein the concentration of bisulfite is between 3.75 M and 6 M.

14. Solution according to any of the claims 12 to 13 wherein the pH value of the solution is 5.5 and wherein the concentration of bisulfite is 5 M.